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Title:

Denaturing strategies for detection of double stranded PCR products on GMR magnetic sensors

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Arrays of GMR magnetic field sensors have been demonstrated for the detection of proteins¹ and DNA². The readout is based on the detection of the target-mediated binding of magnetic nanoparticle (MNP) labels to the sensor surface. The assay is insensitive to the sample matrix as there is virtually no detectable magnetic response from biological samples.

Here, we employ the GMR array platform to detect PCR products from melanoma cell lines, with the final goal of profiling mutations of diagnostic relevance³. The sensor surface is functionalized with ssDNA probes. The forward PCR primers are biotinylated to facilitate binding to streptavidin coated MNPs. The dsDNA product has to be denatured to enable target binding to the sensor surface. We aim to obtain the highest binding signal, as specificity can be increased by optimizing the stringency condition during washing⁴.

In this work, we tested two approaches for the denaturation of PCR products and magnetic labelling:

(1) heat denaturation followed by shock cooling, on-chip hybridization and on-chip labelling with MNPs (**Fig.1a**).

(2) labelling of dsDNA PCR products with MNPs, immobilization of MNPs in a magnetic separation column, denaturation in 6M urea in DI water at 75°C, release of MNPs with ssDNA labels, on-chip detection (**Fig.1b**).

Figure 1c and **Fig.1d** show the GMR signal from experiments using heat denaturation and magnetic separation, respectively. In **Fig.1c** the binding is faster because we measure the biotin-streptavidin binding, whereas the binding in **Fig.1d** is limited by the DNA hybridisation and diffusion of beads with targets. Both methods offer high signals with small deviations. The denaturation in magnetic column results in slightly higher signal due to the complete removal of reverse complement. Both techniques are viable for detection of PCR products on GMR sensors, the choice will be driven by a trade-off between assay time and signal intensity.

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1-Gaster, Richard S., *et al.*, 2009, *Nature medicine* **15**(11) 1327-1332.

2-Xu, Liang, *et al.*, 2008, *Biosensors and Bioelectronics* **24**(1) 99-103.

3-Dahl, Christina, *et al.*, 2013, *Molecular Cancer Research* **11**(10) 1166-1178.

4-Rizzi, Giovanni, *et al.*, 2015, *Journal of Magnetism and Magnetic Materials* **380** 215-220.

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